Assessment of Coronary Stenosis Severity and Transmural Perfusion Gradient by Myocardial Contrast Echocardiography

Comparison of Gray-Scale B-Mode With Power Doppler Imaging

Hisashi Masugata, MD; Bruno Cotter, MD; Barry Peters, MD; Koji Ohmori, MD; Katsufumi Mizushige, MD; Anthony N. DeMaria, MD

Background—The present study (1) compared the ability of power Doppler imaging with that of gray-scale B-mode tissue imaging to opacify the myocardium and detect coronary stenosis by myocardial contrast echocardiography and (2) compared the response of video intensity (VI) to variable pulsing intervals for each modality.

Methods and Results—Four grades of left anterior descending coronary artery (LAD) stenoses were created in 9 open-chest dogs. Stenoses reduced resting LAD flow by 25%, 50%, 75%, and 100% of baseline by flow probe. Myocardial contrast echocardiography was performed during varying ECG gated pulsing intervals (PIs) from 1:1 to 1:10. By gray-scale imaging, background-subtracted LAD bed VI was less than baseline VI at all PIs for the 100% reduced-flow state but not for any other flow state or interval. By power Doppler imaging, LAD bed VI was less than baseline VI at all intervals for 75% and 100% reduced-flow states but only 1:1 and 1:2 for 25% and 50% reduced-flow states, respectively. Correlation of VI and myocardial blood flow (determined by use of fluorescent microspheres) ratios from stenosed versus normal beds was stronger by power Doppler imaging. A transmural opacification gradient with stenosis was visualized and measured by power Doppler imaging, but it was insignificant by gray-scale imaging. The ratio of endocardial/epicardial flow determined by use of fluorescent microspheres was correlated with VI by power Doppler imaging at all PIs.

Conclusions—Power Doppler imaging has advantages compared with gray-scale imaging in opacifying the myocardium and in detecting coronary stenosis and altered transmural distribution of myocardial perfusion from peak VI. Because VI differences from baseline at long PI vary for mild versus severe (75% and 100%, respectively) reduced-flow states, power Doppler imaging may provide a method to quantify coronary stenoses. (Circulation. 2000;102:1427-1433.)

Key Words: echocardiography ■ perfusion ■ blood flow

Although myocardial contrast echocardiography (MCE) can be achieved after intravenous injection of microbubble agents, several technical issues remain uncertain. Controversy exists as to whether gray-scale B-mode tissue imaging or power Doppler recording is the optimal technique to perform MCE. Neither has the relationship between contrast intensity and the pulsing interval of gated images been defined for nonischemic and ischemic segments by either modality. Therefore, the present study was conducted to compare the MCE intensity levels produced by gray-scale B-mode tissue imaging with those observed by power Doppler imaging and to assess the response of each modality to variable image pulsing intervals.

Methods

Animal Preparation

The study was approved by the Animal Research Committee. In 9 open-chest dogs, the heart was suspended in a pericardial cradle, and femoral artery catheters (7F) measured hemodynamics and provided blood samples. The heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. The proximal portion of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissue, and a transit-time flow probe (series 2RB, Transonics System) connected to a digital flowmeter (model T201, Transonics System) was placed snugly around the vessel. A custom-designed screw occluder distal to the flow probe produced graded stenoses.

Myocardial Contrast Echocardiography

Contrast was produced by the continuous infusion of 0.2 mL/min FS-069 (Optison, Molecular Biosystems) with a gently agitated volumetric pump. The dose was selected on the basis of pilot experiments and was the lowest dose that provided definite visible myocardial opacification in gray scale. Gray-scale tissue and power Doppler images were obtained 3 minutes after initiating infusion to ensure that myocardial opacification had reached a plateau intensity. A latex bag filled with degassed saline functioned as an acoustic
interface between the heart and the transducer, which was positioned to image the LAD perfusion territory.

Imaging was performed with a broad-band 4- to 2-MHz transducer (HDI5000, ATL). Second-harmonic (2-MHz transmission, 4-MHz reception), pulse-inversion, 2D gray-scale imaging and color-coded power Doppler recording were performed in the papillary muscle short-axis view during end-systolic ECG triggering. A mechanical index ranging from 1.0 to 1.3 was chosen to obtain optimal myocardial opacification. Pulse repetition frequency was fixed at 1000 Hz for power Doppler imaging. The dynamic range (50 dB), transmitting power, focus, overall gain, image depth, wall filter, and color scale were held constant for each experiment. The interval between the ECG triggers (pulsing interval) was increased from every heart beat (1:1) to every 2 (1:2), 6 (1:6), 8 (1:8), and 10 (1:10) cardiac cycles to allow incremental microbubble replenishment. Five end-systolic images were acquired at each pulsing interval, first by gray-scale imaging and then by harmonic power Doppler imaging. In power Doppler imaging, end-systolic ECG gating was adjusted before contrast injection to minimize the clutter (flash) artifact due to cardiac motion, and the same triggering phase was used in gray-scale imaging. The mechanical index and focus were the same as those in gray-scale imaging, but the images were displayed in color scale.

Images acquired on S-VHS videotape before and after microbubble injection at each pulsing interval were transferred to an offline computer (Macintosh, Apple Computer Inc) and aligned for background subtraction. Images by power Doppler were converted to gray scale by using NIH Image 1.62 on the offline computer. Color was ignored, and only myocardial luminance was assessed. Myocardial video intensity (VI) was measured in gray-scale units ranging from 0 to 255 for both modalities. In this software implementation, myocardial blood flow (MBF) was measured by left atrial injection of 5 × 10^6 10-μm fluorescent microspheres (Molecular Probes) while reference femoral artery samples were withdrawn. After the animal was euthanized, the heart was sliced, and the cross-sectional segment corresponding to the short-axis image was cut into 12 wedge-shaped transmural tissue pieces, each of which was divided into endocardial and epicardial segments. A flow cytometer served to count microspheres. Endocardial and epicardial MBF was calculated from the following equation: Qm = (Cm × Qt)/Cr, where Qm is myocardial segment flow (mL/min), Cm is tissue count, Qt is arterial withdrawal rate (mL/min), and Cr is arterial reference sample count. Transmural MBF (mL·min^-1·g^-1) to 12 wedge-shaped pieces was calculated as the quotient of the summed flows to the individual segments within that piece and their combined weight. MBF to the LAD and LCx beds, defined by Monastral Blue dye (Sigma Chemical Co) injection, was calculated by averaging the transmural MBF in the pieces from each bed, respectively. The ratio of transmural MBF as well as the ratio of endocardial/epicardial MBF was calculated in both LAD and LCx beds.

**Experimental Protocol**

Baseline MCE and MBF were obtained when hemodynamic stability was achieved. Thereafter, by flow probe guidance, the LAD diameter was progressively reduced to produce 4 levels of stenosis, which reduced LAD flow by 25%, 50%, 75%, and 100% of the baseline value. MBF was followed by MBF measurement at each stenosis grade. At the end of the experiment, the LAD was occluded at the occluder site, and Monastral Blue dye was injected into the left atrium to delineate the LAD and LCx beds. The dog was then euthanized, and fluorescent microsphere analysis was completed.

**Statistical Analysis**

Data from all animals were expressed as mean±SD. Comparisons of hemodynamics, MBF, and VI data among the 5 flow states were performed by ANOVA. The difference in VI produced by prolongation of the pulsing interval between gray-scale and power Doppler imaging was tested by paired t test. Correlations between MBF and VI data were performed by linear regression analysis. For differences, a value of P<0.05 was considered significant.

**Results**

**Severity of Coronary Stenosis**

Figure 1 shows measurements of LAD blood flow by flow probe and LAD/LCx ratio of MBF by microsphere analysis for baseline and the 4 levels of stenosis. LAD blood flow and LAD/LCx ratio decreased significantly and progressively from baseline with greater levels of stenosis.

**Visual Differentiation of Severity of Stenosis by MCE**

Visual assessment of gray-scale images (Figure 2) was difficult at the 1:1 pulsing interval because myocardial opacification was weak. The intensity of opacification with the gray scale increased progressively with longer pulsing intervals. Nevertheless, a clear-cut perfusion defect could not
be identified qualitatively with a 25% flow reduction. Visual examination did reveal the presence of diminished perfusion for 75% and 100% reduced-flow states with gating at 1:2 cardiac cycles. However, the area and magnitude of the opacification deficit became progressively smaller with increasing pulsing intervals. Thus, even in the presence of total occlusion, collateral flow was sufficient to opacify some of the at-risk myocardium if sufficient time was provided for microbubbles to enter the area between signal transmissions.

Figure 3 shows images obtained by the power Doppler method. Opacification by power Doppler imaging was greater than that by gray-scale imaging at all pulsing intervals, and all reduced flow states were visualized, even with gating to every cardiac cycle. Because opacification at baseline was nearly complete and intensity was nearly maximal (see below) at a pulsing interval of 1:2 or 1:4, identification of 75% and 100% flow-reduced states was easier with this pulsing interval than with a pulsing interval of 1:1. However, visual recognition of 25% reduced-flow stenosis was more difficult at 1:4 or longer gating than at 1:1 because the VI of the mildly obstructed LAD bed increased markedly in response to prolongation of the pulsing interval. As with gray-scale imaging, the perfusion defect with more marked (75% and 100%) reduced-flow states observed by power Doppler imaging decreased in area and magnitude with incremental prolongation of the pulsing interval. Importantly, because collateral flow filled the at-risk myocardium at longer pulsing intervals, the spatial distribution of the opacification deficit was located in the subendocardial region (Figure 3). Thus, power Doppler imaging provided clear evidence of the predominant subendocardial distribution of perfusion deficits in the presence of coronary stenoses.
Myocardial VI in the Presence of Graded Coronary Stenosis During Varying Pulsing Intervals

VI measurements of the LAD bed with gray-scale imaging progressively increased in proportion to prolongation of the pulsing interval (Figure 4). Although the increase in VI was continuous to a pulsing interval of 1:10, the magnitude of increase tended to be greater from 1:1 to 1:6 and was less marked thereafter. The VIs of the LAD bed at 25%, 50%, and 75% reduced-flow states were not significantly different from baseline or from each other at any pulsing interval in gray-scale imaging. However, total occlusion produced a significant reduction in VI from baseline at all pulsing intervals.

VI measurements with power Doppler imaging also tended to increase progressively with prolongation of the pulsing interval for all flow states, but the increase plateaued at an interval of 1:4 at baseline as well as at the 25% and 50% reduced-flow states. Therefore, although the VI of the LAD bed by power Doppler imaging was significantly decreased compared with baseline for all grades of flow reduction at pulsing intervals of 1:1 and 1:2, these differences became insignificant at pulsing intervals of 1:4 or higher for the 25% and 50% reduced-flow states. Significant differences between baseline and severe (75% and 100%) reduced-flow states continued to be observed at longer pulsing intervals: to 1:8 at 75% and 1:10 at 100%, differentiating these more severe from milder flow-reduced states.

Table 1 shows the correlation between VI and the fluorescence microsphere–derived MBF ratios from the stenosed versus normal bed at varying pulsing intervals. The correlation progressively improved in proportion to prolongation of pulsing interval from 1:1 to 1:10 in gray-scale imaging. The correlation obtained by power Doppler imaging was stronger than that by gray-scale imaging for all pulsing intervals except 1:10. However, the correlation was closest at a pulsing interval of 1:2, decreased gradually, and disappeared at 1:10.

Increase in VI Produced by Prolongation of Pulsing Interval

Figure 5 depicts the change in VI in the LAD bed due to alteration of pulsing intervals from 1:1 to 1:10 at all flow states by both modalities. The increases in the background-subtracted VI during prolongation of pulsing intervals from 1:1 to 1:10 were greater with power Doppler than gray-scale imaging at all flow states. In addition, by gray-scale imaging, the degree of increase in VI in response to a gating change from 1:1 to 1:10 was similar to that of baseline for all flow states, except for total occlusion, which exhibited a lesser magnitude of amplification. Conversely, a significantly greater increase in VI than at baseline was observed by power Doppler imaging for all reduced flow states, with the greatest augmentation seen with the most severe stenoses.

Table 1. Correlation Between VI and MBF Ratios From Stenosed Versus Normal Bed at Varying Pulsing Intervals

<table>
<thead>
<tr>
<th>Pulsing Interval</th>
<th>Gray Scale</th>
<th>Power Doppler</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>1:1</td>
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<td>0.18</td>
</tr>
<tr>
<td>1:2</td>
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<tr>
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<td>0.003</td>
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<td>1:6</td>
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<td>1:8</td>
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<td>0.0005</td>
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<tr>
<td>1:10</td>
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</tbody>
</table>

Figure 4. Background-subtracted VI of stenosed LAD bed at varying pulsing intervals. *P<0.05, **P<0.01, and ***P<0.001 vs baseline; †P<0.05 vs 25%. GL indicates gray levels.

Figure 5. Increase in background-subtracted VI during change in pulsing interval from 1:1 to 1:10 in gray-scale and power Doppler imaging at all flow states. *P<0.001 vs baseline by gray-scale imaging; †P<0.05 and ††P<0.01 vs baseline by power Doppler imaging. GL indicates gray levels.
TABLE 2. Correlation Between VI– and MBF–Endocardial/Epicardial Ratios in LAD Bed at All Flow States During Varying Pulsing Intervals

<table>
<thead>
<tr>
<th>Pulsing Interval</th>
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<th>Power Doppler</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>1:10</td>
<td>0.19</td>
<td>0.22</td>
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</tbody>
</table>

Figure 6. Ratios of endocardial/epicardial VI by power Doppler imaging and MBF analysis at all pulsing intervals in LAD bed at all flow states. MBF indicates myocardial blood flow measured by fluorescent microspheres. *P<0.05, **P<0.01, and ***P<0.001 vs baseline value derived from VI by power Doppler imaging; †P<0.05 and ††P<0.0001 vs baseline value derived from MBF analysis.

Transmural Differences of VI and MBF
A difference in the transmural distribution of opacification with stenosis was easily visualized by power Doppler imaging but was rarely observed by gray-scale imaging (Figures 2 and 3). As expected, with severe flow reductions, there was a decrease in endocardial opacification, with greater preservation of epicardial perfusion and a diminution of the ratio of endocardial to epicardial intensity. By power Doppler imaging, the epicardial side of the LAD bed appeared slightly opacified with severe stenosis (75% and 100% reduced-flow states) during 1:1 ECG gating, and this epicardial opacification became greater at longer pulsing intervals (Figure 3). Figure 6 shows the ratios of endocardial/epicardial VI by power Doppler imaging and microsphere-derived MBF analysis in the LAD bed at all pulsing intervals at all flow states. The alterations in endocardial/epicardial ratio by power Doppler imaging coincided with that obtained by microsphere-derived MBF analysis and was most easily visualized for more severe flow reductions and at longer pulsing intervals. Table 2 shows the correlation between VI– and the microsphere-derived MBF–endocardial/epicardial ratios in the LAD bed at all the flow states during varying pulsing intervals. Although no correlation was observed by gray-scale imaging at any pulsing interval, a significant correlation was observed by power Doppler imaging at all pulsing intervals.

Assessment of Coronary Stenosis by MCE
In this dog model, the intensity of myocardial opacification was consistently greater for power Doppler than gray-scale imaging. MCE with power Doppler imaging correctly de-
picted the progressive reduction of regional MBF produced by graded flow-limiting coronary stenoses. Although the reduction of LAD bed VI at 100% by gray-scale imaging was significant, the reduction of VI observed at other reduced flow states was not, and differentiation between 25%, 50%, and 75% reduced-flow states was impossible. However, a significant reduction of VI was observed at all reduced-flow states by power Doppler imaging, and the difference of VI between 25% and 75% reduced-flow states was significant at 1:2 and 1:4 pulsing intervals (Figure 4). In addition, the correlation between VI and the MBF ratios from the stenosed versus normal bed in the presence of graded coronary stenoses was stronger for power Doppler than gray-scale imaging during 1:1 to 1:8 pulsing intervals. Thus, power Doppler imaging is superior to gray-scale imaging in assessing graded coronary stenoses because of both the denser myocardial opacification produced and the greater VI differences produced by reduced coronary flow.

Different Response of VI to Varying Pulsing Intervals Between Gray-Scale and Power Doppler Imaging

In gray-scale imaging, LAD bed VI tended to increase slightly with each prolongation of the pulsing interval from 1:1 to 1:10 at all flow states (Figure 4). In contrast, VI assessed by power Doppler imaging plateaued at a pulsing interval of 1:4 at baseline and at 25% and 50% reduced-flow states but continued to increase to an interval of 1:10 at 75% and 100% reduced flow. This difference was likely due to the fact that the number of microbubbles in the imaging field with normal or mildly decreased coronary flow was sufficient to saturate the power Doppler signal even at a relatively short pulsing interval but was insufficient to saturate in the presence of severe stenosis even at long pulsing intervals. Thus, the use of varying pulsing intervals may provide useful information regarding the severity of stenosis: both mild and severe stenoses can produce abnormalities with a short pulsing interval, whereas only severe stenoses can produce perfusion defects with a long pulsing interval. Such analysis may provide a potential criteria by which to identify stenoses by clinical MCE.

As opposed to previous gray-scale B-mode studies, which reported that the increase in VI with prolonged pulsing interval was blunted in the presence of coronary artery disease, we observed a greater augmentation in VI with stenosis by using power Doppler imaging. This finding was demonstrated by comparing the change of LAD bed VI to incremental pulsing intervals from 1:1 to 1:10 for the 2 imaging methods (Figure 5). The increase in VI for the 2 techniques during prolongation of the pulsing interval from 1:1 to 1:10 was similar at baseline. However, in the presence of coronary stenoses, the VI increase was less than that at baseline when gray-scale imaging was used but greater than that at baseline when power Doppler imaging was used. Thereby, prolongation of the pulsing interval to 1:10 was effective in identifying coronary stenoses by the gray-scale method because a greater increase in VI occurred at baseline than at any reduced flow state. Conversely, the prolongation of pulsing interval produced a greater increase in VI at reduced flow states than at baseline as assessed by power Doppler imaging, which diminished the VI difference between stenosed and normal beds. This difference between the 2 imaging methods is in agreement with the finding that the correlation between VI- and MBF-derived ratios from the stenosed versus normal bed progressively improved in proportion to prolongation of the pulsing interval from 1:1 to 1:10 by gray-scale imaging but was closest at 1:2 and disappeared at 1:10 by power Doppler imaging (Table 1).

The difference between the 2 imaging modalities also influenced the definition of the risk area at various pulsing intervals. The mean value of the risk area of an occluded coronary artery expressed as a percentage of the left ventricular short-axis area was 48±8% by Monastral Blue staining. The mean value of the risk area at the 1:1 pulsing interval was 33±3% by gray-scale imaging and 38±13% by power Doppler imaging (P=NS), and at the 1:10 pulsing interval, it was 20±6% by gray-scale imaging and 14±6% by power Doppler imaging (P<0.01). Thus, both imaging modalities underestimated the risk area at all pulsing intervals. However, the underestimation of risk area during long pulsing intervals was slightly greater by power Doppler than by gray-scale imaging, whereas that during short pulsing intervals was greater by gray-scale imaging.

Transmural Distribution of Myocardial Perfusion

Controversy continues regarding the ability of MCE to determine the ratio of endocardial/epicardial MBF. In a carefully performed recent study using mathematical analysis of microbubble destruction, Linka et al demonstrated good correlation between MCE-derived and microsphere-derived endocardial/epicardial MBF ratio values. In the present study, identification of disturbed transmural distribution of myocardial perfusion by simple visual examination was uncertain with gray-scale imaging but was possible with power Doppler imaging. Abnormal endocardial/epicardial ratios were particularly apparent with severe stenosis (75% and 100% reduced-flow states) and during long pulsing intervals (1:4 or greater) (Figure 3). In addition, the ratio of endocardial/epicardial VI derived from power Doppler at baseline and all reduced flow states correlated with the ratio of endocardial/epicardial MBF measured by microspheres (Table 2). The correlation between the endocardial/epicardial ratios by MCE and microsphere-derived MBF in the present study was not as close as the data that included velocity of microbubble filling reported by Linka et al. This difference suggests that peak VI combined with the velocity of microbubbles will be superior to peak VI alone for quantification of flow. However, power Doppler imaging can identify low levels of flow in the epicardial region in the presence of stenosis. Our data clearly establish that the greater sensitivity of the power Doppler than the gray-scale method to image-contrast microbubbles translates to a superior capability to delineate endocardial/epicardial blood flow, despite lower spatial resolution. This ability to depict transmural distribution of myocardial perfusion may be of value in the detection and quantitative assessment of coronary stenoses by clinical MCE.
Methodological Considerations

Although the present study was performed in open-chest dogs, we believe the principles established remain valid. The experiments used one instrument and one contrast agent, but the properties of each are relatively generic. The dose of the agent used saturated the imaging field during some pulsing intervals and flow states with power Doppler imaging. Although the precise results obtained with other doses might differ, these data have established the basis for the interaction of the pulsing interval and lesion severity in power Doppler recordings. Finally, in the protocol, we examined only stenoses with resting flow reductions in an anterior location and did not apply vasodilator stimuli. Because resting MBF is affected only by stenoses that are >85% in diameter, the differences in cross-sectional LAD area among the reduced flow states in the present study were likely smaller than the non–flow-limiting stenoses in previous studies. This may explain why the differentiation among 25%, 50%, and 75% reduced-flow states was limited in gray-scale images. Nevertheless, we established the feasibility of and criteria for evaluating graded stenoses by MCE at rest, principles that should be applicable to lesions that do not impede resting flow and are best studied with vasodilator stress.

Clinical Implications

We believe that our data demonstrate potential advantages of power Doppler over gray-scale B-mode imaging. Higher myocardial intensity levels are achieved, perfusion defects are more readily identified both visually and quantitatively, and disturbed endocardial to epicardial ratios can be identified by power Doppler imaging. As was evident in the present study, MCE perfusion defects could be visually absent under certain imaging conditions, even in the presence of total arterial occlusion. We believe that our findings support the need to derive quantitative measures of contrast intensity to accurately assess myocardial perfusion. Including microbubble velocity in addition to peak VI may provide more accurate assessment of coronary stenosis and transmural distribution of MBF in power Doppler and gray-scale imaging. Our data indicate that peak background-subtracted VI from power Doppler imaging compared with gray-scale imaging provides new and different information for the assessment of regional microcirculatory flow.

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References

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